```
d his

     (FILE 'HOME' ENTERED AT 17:08:17 ON 08 OCT 2002)

     FILE 'MEDLINE' ENTERED AT 17:08:25 ON 08 OCT 2002
L1            68 S REP AND CAP
L2         18575 S ADENOVIRUS
L3         33096 S HERPESVIRUS
L4             2 S L1 AND L2 AND L3
                 E HELIBRONN R/AU
                 E HEILBRONN R/AU
L5            21 S E3
L6             0 S L3 AND L1 AND L5
L7             1 S L3 AND  L5
```

Promoter Regions (Genetics)
Replication Origin
Transfection
*Viral Proteins: GE, genetics
Viral Proteins: ME, metabolism
*Virus Replication
RN **137750-19-7 (human adeno-associated virus type-2 rep proteins)**
CN 0 (DNA, Recombinant); 0 (DNA, Viral); 0 (DNA-Binding Proteins); 0 (Genetic Vectors); 0 (Viral Proteins)

L5 ANSWER 3 OF 3 MEDLINE
AN 97200259 MEDLINE
DN 97200259 PubMed ID: 9048194
TI Lack of site-specific integration of the recombinant **adeno-associated virus** 2 genomes in human cells.
AU Ponnazhagan S; Erikson D; Kearns W G; Zhou S Z; Nahreini P; Wang X S; Srivastava A
CS Department of Medicine, Indiana University School of Medicine, Indianapolis 46202-5120, USA.
NC AI-26323 (NIAID)
HL-48342 (NHLBI)
HL-53586 (NHLBI)
+
SO HUMAN GENE THERAPY, (1997 Feb 10) 8 (3) 275-84.
Journal code: 9008950. ISSN: 1043-0342.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199708
ED Entered STN: 19970902
Last Updated on STN: 19990129
Entered Medline: 19970821
AB The **adeno-associated virus** 2 (AAV)-based vector system has been suggested for its potential use in human gene therapy because the wild-type (wt) AAV genome appears to integrate into the human chromosomal DNA in a site-specific manner. We systematically investigated the integration patterns of the recombinant AAV genomes lacking one or both the viral coding sequences. Four recombinant AAV genomes were constructed containing the genes for resistance to tetracycline (TcR) and the **herpesvirus** thymidine kinase (TK) promoter-driven gene for resistance to neomycin (neoR; vTc.Neo), the genes for resistance to ampicillin (ApR) and TK-neoR (vAp.Neo), the genes for AAV replication (**rep**) genes and TK-neoR (vRep.Neo), and the AAV capsid (**cap**) genes and TK-neoR (vCap.Neo). The integration pattern of each of the recombinant AAV genomes in individual clonal isolates of the human nasopharyngeal carcinoma cell line (KB) analyzed on Southern blots using a neo-specific DNA probe was distinctly different. In addition, in none of the clones examined was the proviral genome covalently linked to the previously described AAV right-junction (Rt.Jn.) human chromosomal DNA fragment, the putative specific-site of integration for the wt AAV genome. Furthermore, whereas a 276-bp DNA fragment could be readily amplified from each of these clones, using a neo-specific primer-pair by polymerase chain reaction (PCR), no amplified DNA product was obtained using the neo- and the Rt.Jn. primer-pair under identical conditions. Fluorescence in situ hybridization (FISH) analyses further revealed the lack of integration of the recombinant AAV into human chromosome 19, even in the presence of a functional **rep** gene as determined by rescue of the recombinant AAV genome in the presence of adenovirus. These data suggest that the recombinant AAV genomes integrate at sites that are different from that characterized for the wt AAV genome. These studies may have implications in the development of the AAV-based

vector system for its potential use in human gene therapy.

CT Check Tags: Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
*DNA Transposable Elements: GE, genetics
DNA, Recombinant: IP, isolation & purification
*Dependovirus: GE, genetics
Dependovirus: IP, isolation & purification
Genetic Vectors: GE, genetics
Genome, Viral
*Recombination, Genetic: GE, genetics
Tumor Cells, Cultured
*Virus Integration

CN 0 (DNA Transposable Elements); 0 (DNA, Recombinant); 0 (Genetic Vectors)

=> d l5 1-3 all

L5 ANSWER 1 OF 3 MEDLINE
AN 2001464622 MEDLINE
DN 21400689 PubMed ID: 11509889
TI Characterization of permanent cell lines that contain the AAV2 **rep -cap** genes on an Epstein-Barr-virus-based episomal plasmid.
AU Neyns B; Vermeij J; Teugels E; De Rijcke M; Hermonat P; De Greve J
CS Laboratory of Medical Oncology, Oncologisch Centrum, Akademisch Ziekenhuis, Vrije Universiteit Brussel, Laarbeeklaan 101, B-1090 Brussels, Belgium.
SO INTERVIROLOGY, (2001) 44 (4) 255-63.
Journal code: 0364265. ISSN: 0300-5526.
CY Switzerland
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200110
ED Entered STN: 20010820
Last Updated on STN: 20011015
Entered Medline: 20011011
AB Recombinant **adeno-associated virus** (rAAV) has emerged as a promising gene therapy vector. Its development, however, has been hampered by the lack of a readily available efficient production method. We investigated the possibility of establishing permanent cell lines for the production of rAAV with a new Epstein-Barr-virus (EBV)-based episomal AAV **rep-cap** plasmid (pCEP-**rep/cap**). HeLa and 293 cells were stably transfected with plasmids that carry the AAV2 **rep/cap** genes under transcriptional control of their endogenous promoters (p5, p19 and p40) either on the pCEP-**rep/cap** or an integrated (pIM45) plasmid. For the ease of monitoring transgene expression in live cells, a rAAV vector expressing gfp (the green fluorescent protein gene, rAAV-gfp/neo) was used. Establishment of stable transfected cell lines with these plasmids proved feasible but their usefulness was limited because of their instability. Within 8-12 weeks after their establishment, stably transfected **rep-cap** cell lines invariably lost their function. In addition, the rAAV-gfp/neo vector we used was susceptible to mutation in stably transfected HeLa cells. Our observations demonstrate specific problems both at the level of **rep/cap** gene function and the rAAV genome that can occur with the establishment of rAAV production cell lines. These experiments should aid the further development of efficient rAAV production protocols.

CT Check Tags: Human; Support, Non-U.S. Gov't
Cell Line
*Dependovirus: GE, genetics
Gene Therapy: MT, methods
*Genes, Viral
Hela Cells
***Herpesvirus 4, Human: GE, genetics**
Luminescent Proteins: GE, genetics
Plasmids
*Transfection: MT, methods
RN 147336-22-9 (green fluorescent protein)
CN 0 (Luminescent Proteins); 0 (Plasmids)

L5 ANSWER 2 OF 3 MEDLINE
AN 1998001403 MEDLINE
DN 98001403 PubMed ID: 9343238
TI Recombinant **adeno-associated virus** type 2

replication and packaging is entirely supported by a herpes simplex virus type 1 amplicon expressing **Rep** and **Cap**.

AU Conway J E; Zolotukhin S; Muzyczka N; Hayward G S; Byrne B J
CS Department of Pharmacology and Molecular Science, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA.

NC RO1 CA28473 (NCI)
T32 CA 09243 (NCI)
SO JOURNAL OF VIROLOGY, (1997 Nov) 71 (11) 8780-9.
Journal code: 0113724. ISSN: 0022-538X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199711
ED Entered STN: 19971224
Last Updated on STN: 19971224
Entered Medline: 19971113

AB Recombinant **adeno-associated virus** (AAV) type 2 (rAAV) vectors have recently been shown to have great utility as gene transfer agents both in vitro and in vivo. One of the problems associated with the use of rAAV vectors has been the difficulty of large-scale vector production. Low-efficiency plasmid transfection of the rAAV vector and complementing AAV type 2 (AAV-2) functions (**rep** and **cap**) followed by superinfection with adenovirus has been the standard approach to rAAV production. The objectives of this study were to demonstrate the ability of a recombinant herpes simplex virus type 1 (HSV-1) amplicon expressing AAV-2 **Rep** and **Cap** to support replication and packaging of rAAV vectors. HSV-1 amplicon vectors were constructed which contain the AAV-2 **rep** and **cap** genes under control of their native promoters (p5, p19, and p40). An HSV-1 amplicon vector, HSV-RC/KOS or HSV-RC/d27, was generated by supplying helper functions with either wild-type HSV-1 (KOS strain) or the ICP27-deleted mutant of HSV-1, d27-1, respectively. Replication of the amplicon stocks is not inhibited by the presence of AAV-2 **Rep** proteins, which highlights important differences between HSV-1 and adenovirus replication and the mechanism of providing helper function for productive AAV infection. Coinfection of rAAV and HSV-RC/KOS resulted in the replication and amplification of rAAV genomes. Similarly, rescue and replication of rAAV genomes occurred when rAAV vector plasmids were transfected into cells followed by HSV-RC/KOS infection and when two rAAV proviral cell lines were infected with HSV-RC/KOS or HSV-RC/d27. Production of infectious rAAV by rescue from two rAAV proviral cell lines has also been achieved with HSV-RC/KOS and HSV-RC/d27. The particle titer of rAAV produced with HSV-RC/d27 is equal to that achieved by supplying **rep** and **cap** by transfection followed by adenovirus superinfection. Importantly, no detectable wild-type AAV-2 is generated with this approach. These results demonstrate that an HSV-1 amplicon expressing the AAV-2 genes **rep** and **cap** along with HSV-1 helper functions supports the replication and packaging of rAAV vectors in a scaleable process.

CT Check Tags: Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Cloning, Molecular
*DNA Replication
DNA, Recombinant
DNA, Viral: GE, genetics
DNA-Binding Proteins: GE, genetics
*Dependovirus: GE, genetics
Gene Transfer Techniques
Genes, Structural, Viral
*Genetic Vectors: GE, genetics
Hela Cells
***Herpesvirus 1, Human: GE, genetics**